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RESEARCH ARTICLE

CHROMOSOMAL STUDY OF MUTAGENESIS INDUCED BY GAMMA RAYS ON BONE MARROW CELLS OF HOUSE MUSK SHREW *SUNCUS MURINUS*

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SUMMARY Ionizing radiations such as X rays and gamma rays are used in nuclear reactors, spaceships, cancer therapy, taking images of internal bones, etc. but are detrimental to living cells and tissues. In the present investigation, 2 different doses of gamma radiations (0.8 Gy and 2.40 Gy) were used on bone marrow cells of house musk shrew, *Suncus murinus*. The animals were subjected to whole body γ-irradiation exposure in 2 different sets. Seven different types of chromosomal aberrations were scored from bone marrow cells of the shrew, after 4 different time intervals post γ-exposure, at 1 h, 16 h, 48 h and 1 wk (168 h). As a result, in case of both 0.8 Gy and 2.4 Gy exposures, the maximum percentage of aberrations had been obtained after 16 h of exposure (9.06% in case of 0.8 Gy and 12.38% in case of 2.4 Gy) while minimum was observed after 1 wk of exposure. On the other hand, when compared among the types of aberrations, the maximum frequency of aberrations in mitotic chromosomes were observed with centromeric dissociation for both the doses of γ rays (1.97% in case of 0.8 Gy and 2.90% in case of 2.4 Gy), while ring chromosomes scored the minimum. Further, statistical analysis of the data reveals that the aberrations are non-random in distribution. They are somewhat time-dependent and centromeric regions of some chromosomes are most vulnerable to γ irradiation.

Keywords: *Suncus murinus,* gamma radiation, chromosomal aberrations, mitotic chromosomes.

INTRODUCTION

Chromosomal aberrations had been studied in wide varieties of animals such as mice, fish, grasshoppers, monkeys, and even in plants. Pioneering work by Muller (1927) on artificial mutagenesis in *Drosophila melanogaster* and development of *ClB* technique paved the path for study of the effect of radiations on chromosomes (Gardner & Snustad, 1984). Later, innumerable researchers had worked in such domain and some notable works are, Krause & Ziegler (1906) on mammalian tissue, Amato (1911) and Grasnick (1918) on amphibian eggs and larva, Mohr (1918) and Stadler (1928) on maize chromosomes, Goodspeed & Olson (1928) on *Nicotiana* sp. Other significant workers in this field are Carlson (1938, 1940, 1941a, 1941b), Carlson et al. (1949), Manna & Mazumder (1962, 1967, 1968), Mazumder & Manna (1967) and Holleander (1964). Even work on common Rhesus monkey, *Macaca mulatta* had been pursued by Koshi-chenko & Semenov (1975) and Van & Paul (1976). Sur & Manna (1987) had studied the effect of X rays on grasshopper chromosomes, Sarkar & Manna (2001) studied the effect of nitrogen fixing bacterium, *Beijerinckia indica* on 3 mammalian models i.e. shrew, monkey and cat. Sur (2004) had reported the effect of X rays and

chemicals on 2 different species of grasshopper.

It has been found in mice that chronic irradiation induces a somewhat fewer mutations than the same dose of acute irradiation. Moreover, on treatment with intermittent dose of a specific irradiation in mice, it was found to induce a slightly lower mutation frequency, when compared with continuous dose of the same irradiation (Gardner & Snustad 1984). The single-hit theory means that one ionization can produce one mutation, but this does not inform anything about the efficiency of the process (Gardner & Snustad 1984). Chromosomal aberrations are induced more frequently in metaphase stage, than at the interphase (Gardner & Snustad 1984), fetching structural changes as deletions, translocations, duplication and inversions.

Suncus murinus belongs to the family Soricidae (Manna & Talukdar 1967). In the present study, metaphase stage of bone marrow cells from femur bone of *S. murinus* is chosen, due to its rapidly proliferating and spontaneously dividing nature, hematopoietic character, dividing mitotically as well as availability of easy standard preparation technique.

In our previous studies, we have extensively studied and reported genotoxic effects of X rays and gamma rays on meiotic chromosomes in laboratory animal models such as different species of grasshoppers, and mitotic chromosomes in fish and mice (Das 2021, Sur et al. 2017, Sur et al. 2010, Sur & Das 2012a, 2012b). The study on effect of radiation on chromosomes of wild animal shrew (*S. murinus*) is limited. The present study had been undertaken to meet such objective. The work had been cleared by Animal Ethical Committee of University of Kalyani, Kalyani, West Bengal.

MATERIALS AND METHODS

Collection of animals and maintenance

Adult healthy house musk shrew (*S. murinus*) of both sexes were caught from their natural habitats like hedges, near drains, human dwellings, under concrete slabs, etc. during night, when the animals come out of their dwellings in search of food. The animals were transferred to laboratory and maintained for a short period, with food and water.

Radiation

After maintenance, the animals were subjected to whole body γ irradiation of 2 different doses i.e. 0.8 Gy (80 rad) and 2.4 Gy (240 rad) from $_{27}Co^{60}$ γ ray source. The shrews were caged in special container and subjected to γ ray in sets of 3 shrews for each h (Table 1).

After exposure, the shrews were transferred to the laboratory. They were chloroformed and sacrificed after 4 different time intervals of 1 h, 16 h, 48 h and 1 wk (the shrew did not live after 1 wk of exposure). Mitotic chromosomes of the animals were studied from well spread metaphase plates prepared from femur bone marrow cells of the hind legs. The bone marrow was suspended in 1% sodium citrate solution (hypotonic solution) and was flushed to bring the cells into a uniform suspension. Then cells were fixed in acetic-alcohol (1:3 v/v). The slides were prepared by dropping the cells on chilled slides maintained at –5° C. Staining was done by flooding the slides with Giemsa staining solution for one and a half h. Data was scored by using 300 cells for each time interval. Analysis of data was done by using different types of standard statistical tools. A total of 48 shrews were taken as the test series for both the doses, while the same number of individuals served as control (Table 1).

TABLE 1: Number of shrews (male and female) irradiated with γ ray dose 0.80 Gy and 2.4 Gy and their bone-marrow cells fixed at different time intervals.

Fixation time	γ ray 0.8 Gy dose				γ ray 2.4 Gy dose			
	No. of males	No. of females	No. in total	No. died	No. of males	No. of females	No. in total	No. died
1 h	3	3	6	-	3	3	6	-
16 h	3	3	6	-	3	3	6	1
48 h	3	3	6	-	3	3	6	-
1 wk (168 h)	3	3	6	2	3	3	6	3

OBSERVATIONS

For 0.8 Gy γ-ray dose

Control series

A total of 300 mitotic cells for each h, summing up to 1200 cells at 4 different time intervals were studied in this series. The diploid chromosome number is 40. Therefore, for 1200 cells a total of 48,000 mitotic chromosomes were studied in this whole control series. The maximum chromosomal aberrations were observed with centromeric dissociation (0.15%), while minimum aberrations were obtained with ring chromosome (0.02%). Therefore, no significant aberrations were found in this control series (Table 2).

Treated series

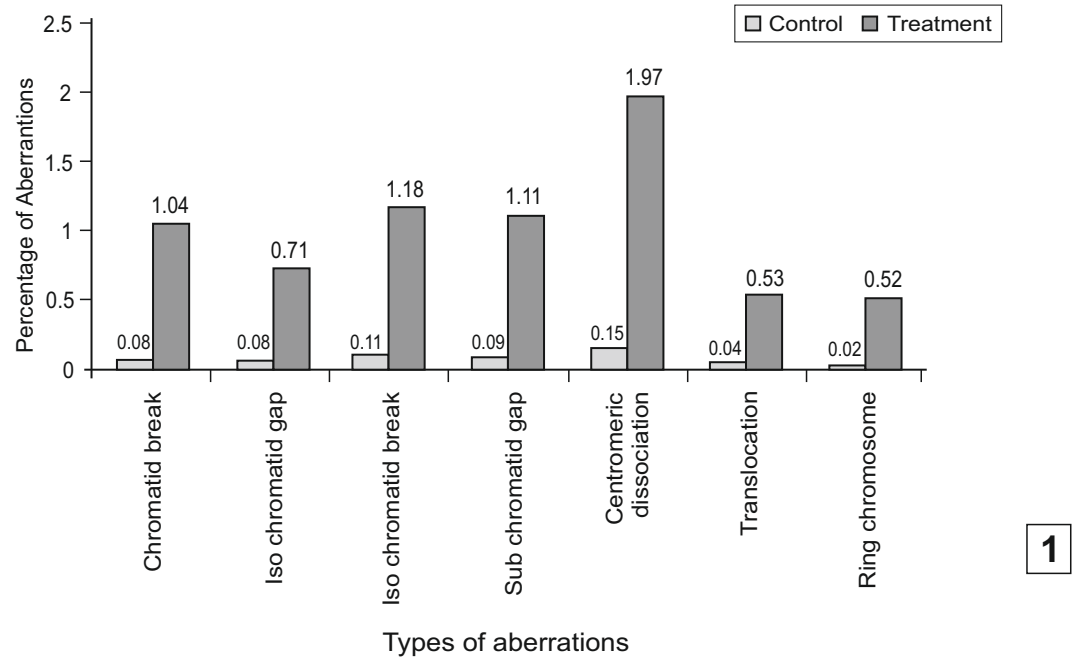
In this series, a total of 1200 cells and 48000 chromosomes were studied. Examination of chromosomes after γ irradiation showed that the rays were capable of inducing 7 different types of chromosomal aberrations, as chromatid breaks, iso-chromatid gap, iso-chromatid break, sub-

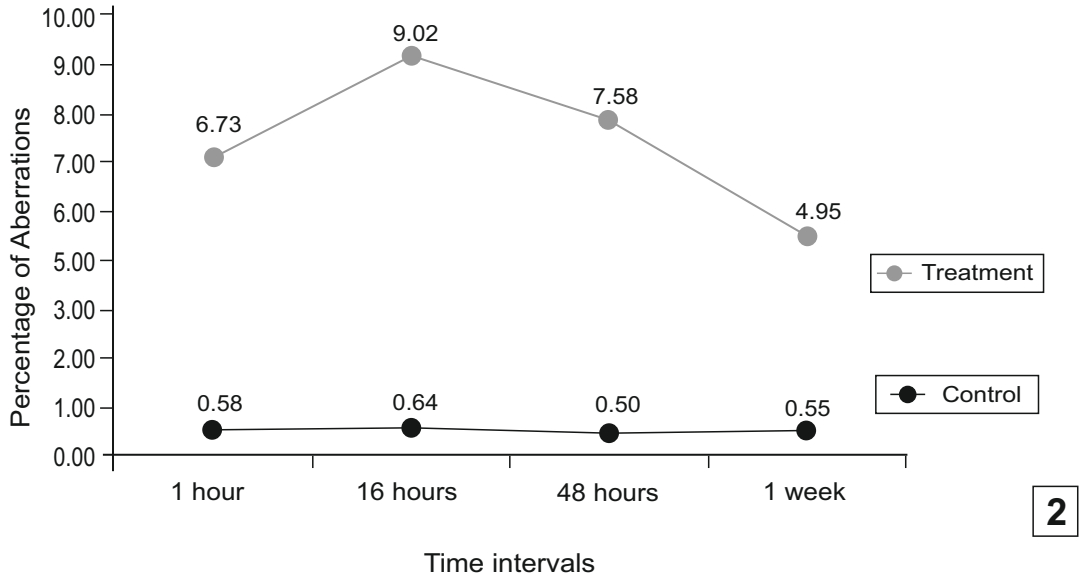
TABLE 2: γ ray (0.8 Gy) irradiated chromosomal aberrations in treated and control shrews.

Time		No. of cells	No. of chromosomes	Types of aberrations: Chromatid break	iso-chromatid gap	iso-chromatid break	Sub-chromatid gap	Centromeric dissociation	Trans-location	Ring chromosome	Total aberrations	Total aberration %
1 h	C	300	12000	3	15	18	12	15	2	4	69	**0.58**
	T	300	12000	102	98	186	98	201	68	54	807	**6.73**
16 h	C	300	12000	11	7	3	23	26	5	2	77	**0.64**
	T	300	12000	147	98	163	156	385	72	61	1082	**9.02**
48 h	C	300	12000	11	8	18	2	12	8	1	60	**0.50**
	T	300	12000	155	105	142	174	195	68	71	910	**7.58**
1 wk	C	300	12000	15	7	12	8	17	3	4	66	**0.55**
	T	300	12000	96	42	73	107	164	48	64	594	**4.95**
TA	C	**1200**	**48,000**	**40**	**37**	**51**	**45**	**70**	**18**	**11**	**272**	**0.57**
	T	**1200**	**48,000**	**500**	**343**	**564**	**535**	**945**	**256**	**250**	**3393**	**7.07**
Total aberration %			C	**0.08**	**0.08**	**0.11**	**0.09**	**0.15**	**0.04**	**0.02**		
			T	**1.04**	**0.71**	**1.18**	**1.11**	**1.97**	**0.53**	**0.52**		

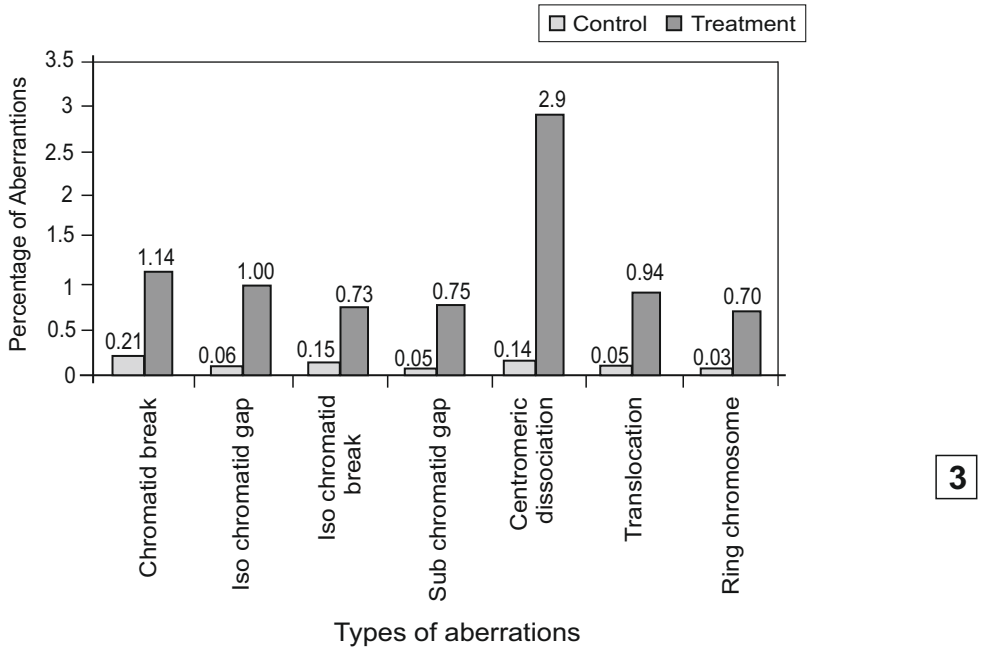
abrr, aberrations; C, Control series; T, Treated series; TA, Total aberrations

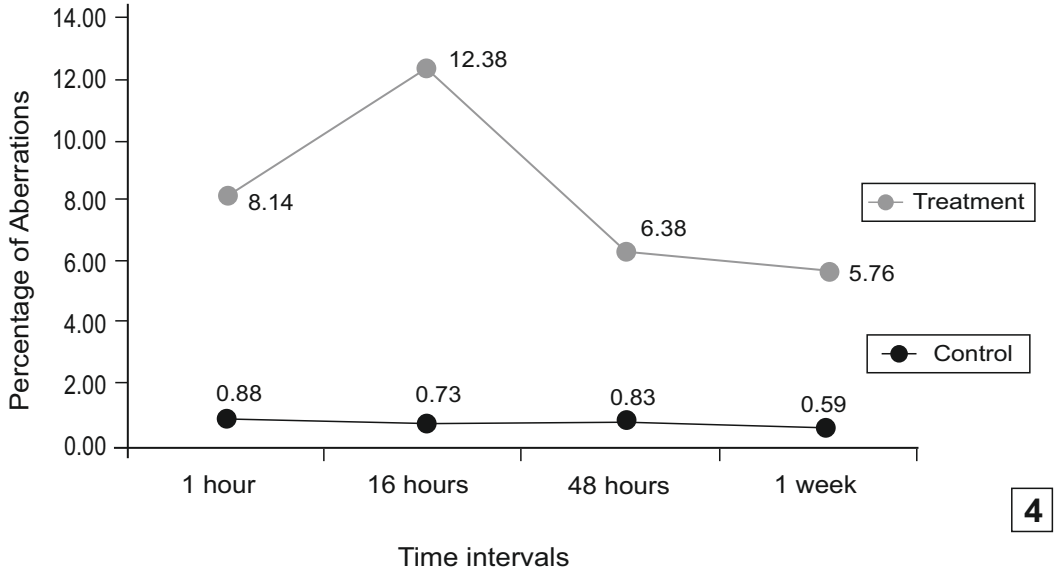
DAS:





Figs 1 & 2: 1. Bar-diagram showing seven different types of chromosomal aberrations. 2. Line-diagram showing chromosomal aberrations with respect to time in bone marrow cells of shrew induced by γ ray (0.8 Gy) and compared with control.





Figs 3 & 4: 3. Bar-diagram showing seven different types of chromosomal aberrations. 4. Line-diagram showing chromosomal aberrations with respect to time in bone marrow cells of shrew induced by γ ray (2.4 Gy) and compared with control.

DAS:

TABLE 3: γ-ray (2.4 Gy) irradiated chromosomal aberrations in treated and control shrews.

Time		No. of cells	No. of chromo-some	Types of aberrations: Chromatid break	iso-chromatid gap	iso-chromatid break	Sub-chromatid gap	Centromeric dissociation	Trans-location	Ring chromo-some	Total aberrations	Total aberration %
1 h	C	300	12000	25	9	20	8	18	18	7	105	**0.88**
	T	300	12000	140	165	88	59	370	75	80	977	**8.14**
16 h	C	300	12000	28	7	15	6	16	12	4	88	**0.73**
	T	300	12000	129	183	161	172	485	196	159	1485	**12.38**
48 h	C	300	12000	23	5	26	4	24	8	9	99	**0.83**
	T	300	12000	145	75	46	56	273	85	85	765	**6.38**
1 wk	C	300	12000	25	17	9	5	10	3	2	71	**0.59**
	T	300	12000	135	55	53	75	265	96	12	691	**5.76**
TA	C	**1200**	**48,000**	**101**	**38**	**70**	**23**	**68**	**41**	**22**	**363**	**0.76**
	T	**1200**	**48,000**	**549**	**478**	**348**	**362**	**1393**	**452**	**336**	**3918**	**8.16**
Total aberration %			C	**0.21**	**0.08**	**0.15**	**0.05**	**0.14**	**0.09**	**0.05**		
			T	**1.14**	**1.00**	**0.73**	**0.75**	**2.90**	**0.94**	**0.70**		

abrr, aberrations; C, Control series; T, Treated series; TA, Total aberrations.

TABLE 4: Statistical analysis of effect of γ rays (0.8 Gy) on mitotic chromosomes in shrew at different time intervals.

Aberration statistics	Chromatid break	Iso-chromatid gap	Iso-chromatid break	Sub-chromatid gap	Centromeric dissociation	Translocation	Ring chromosome	Total aberration
Control S.E. +	**2.52**	**1.93**	**3.54**	**4.42**	**3.01**	**1.32**	**0.75**	**3.54**
Treatment S.E.+	**15.15**	**14.68**	**24.38**	**18.50**	**50.24**	**5.42**	**3.57**	**102.94**
Control C.D. at 5%	**4.93**	**3.78**	**6.95**	**8.67**	**5.91**	**2.59**	**1.47**	**6.93**
Treatment C.D. at 5%	**29.63**	**28.77**	**47.79**	**36.27**	**98.47**	**10.62**	**6.99**	**201.76**
Control C.D. at 1%	**6.48**	**4.97**	**9.13**	**11.39**	**7.76**	**3.41**	**1.93**	**9.10**
Treatment C.D. at 1%	**39.01**	**37.79**	**62.78**	**47.65**	**129.37**	**13.95**	**9.19**	**265.06**
t - Values	**7.49****	**5.17****	**5.21****	**6.44****	**4.35****	**10.67****	**16.73****	**7.59****
Chi-Square Values	**10.16****	**4.40**	**15.49****	**18.66****	**2.69**	**3.87**	**4.16**	**9.81***
r - Values	**0.15**	**0.34**	**0.08**	**-0.05**	**0.89****	**0.37**	**0.82****	**0.49**

Overall F value = 15.90 **; * Significant at 5% level; ** Significant at 1% level.

Standard analysis of the data were undertaken as Standard error (S.E) ; Critical difference (C.D) at 1% and 5 % levels; t- test ; chi- square analysis and correlation coefficient (r-value) and Analysis of Variance (F-Value) of both treatment and control species.

chromatid gap, centromeric dissociation, trans-location and ring chromosomes (Figs 5–7). Out of these different types of aberrations, maximum frequency of aberrations was obtained with centromeric dissociation (1.97%) while the minimum was obtained with ring chromosome (0.52%) (Table 2, Fig. 1).

When compared with respect to time, it was found out that chromosomal aberration increased slowly from 1h (6.73%) to 16 h post exposure to γ ray, was maximum at 16th h (9.02%), and then decreases and becomes negligible after 1 wk of exposure (4.95%) (Table 2, Fig. 2). Therefore, a total of 7.07% of chromosomal aberrations had

TABLE 5: Statistical analysis of effect of γ rays (2.4 Gy) on mitotic chromosomes in shrew at different time intervals.

Aberration statistics	Chromatid break	Iso-chromatid gap	Iso-chromatid break	Sub-chromatid gap	Centromeric dissociation	Translocation	Ring chromosome	Total aberration
Control S.E. +	**1.03**	**2.63**	**3.62**	**0.85**	**2.89**	**3.17**	**1.55**	**7.47**
Treatment S.E.+	**3.42**	**31.94**	**26.32**	**27.48**	**51.45**	**28.00**	**19.46**	**170.66**
Control C.D. at 5%	**2.02**	**5.15**	**7.09**	**1.67**	**5.66**	**6.22**	**3.05**	**14.63**
Treatment C.D. at 5%	**6.71**	**62.61**	**51.59**	**53.87**	**100.84**	**54.87**	**38.14**	**334.49**
Control C.D. at 1%	**2.65**	**6.77**	**9.31**	**2.20**	**7.43**	**8.17**	**4.00**	**19.22**
Treatment C.D. at 1%	**8.82**	**82.25**	**67.78**	**70.27**	**132.49**	**72.09**	**50.11**	**439.44**
t - Values	**4.25****	**3.43****	**2.62***	**3.08****	**6.45****	**3.65****	**4.88****	**5.30****
Chi-Square Values	**1.24**	**36.24****	**28.08****	**6.69***	**10.46****	**18.68****	**7.38***	**29.33****
r - Values	**-0.95**	**-0.46**	**-0.21**	**0.08**	**-0.03**	**0.03**	**-0.27**	**0.08**

Over all F value = 42.31**; * Significant at 5% level; ** Significant at 1% level

Standard analysis of the data were undertaken as Standard error (S.E) ; Critical difference (C.D) at 1% and 5 % levels; t- test ; chi- square analysis and correlation coefficient (r-value) and Analysis of Variance (F-Value) of both treatment and control species.

been scored in this treated series (Table 2).

Statistical analysis of the data reveals that the χ^2 (Chi-square) value for the treated series are 10.16 for chromatid break, 15.49 for iso-chromatid break, 18.66 for subchromatid gap. These values are highly significant. The overall χ^2 value for the pooled data is 9.81. The overall F-value is 15.90 which is highly significant (Table 4).

For 2.4 Gy γ ray dose

Control series

In this series, 1200 bone marrow cells and therefore 48000 mitotic chromosomes were studied. Negligible chromosomal aberrations had been observed herein, highest being 0.21% with chromatid break and lowest being 0.05% with ring chromosome and subchromatid gap. Therefore, negligible aberrations were noticed here (Table 3).

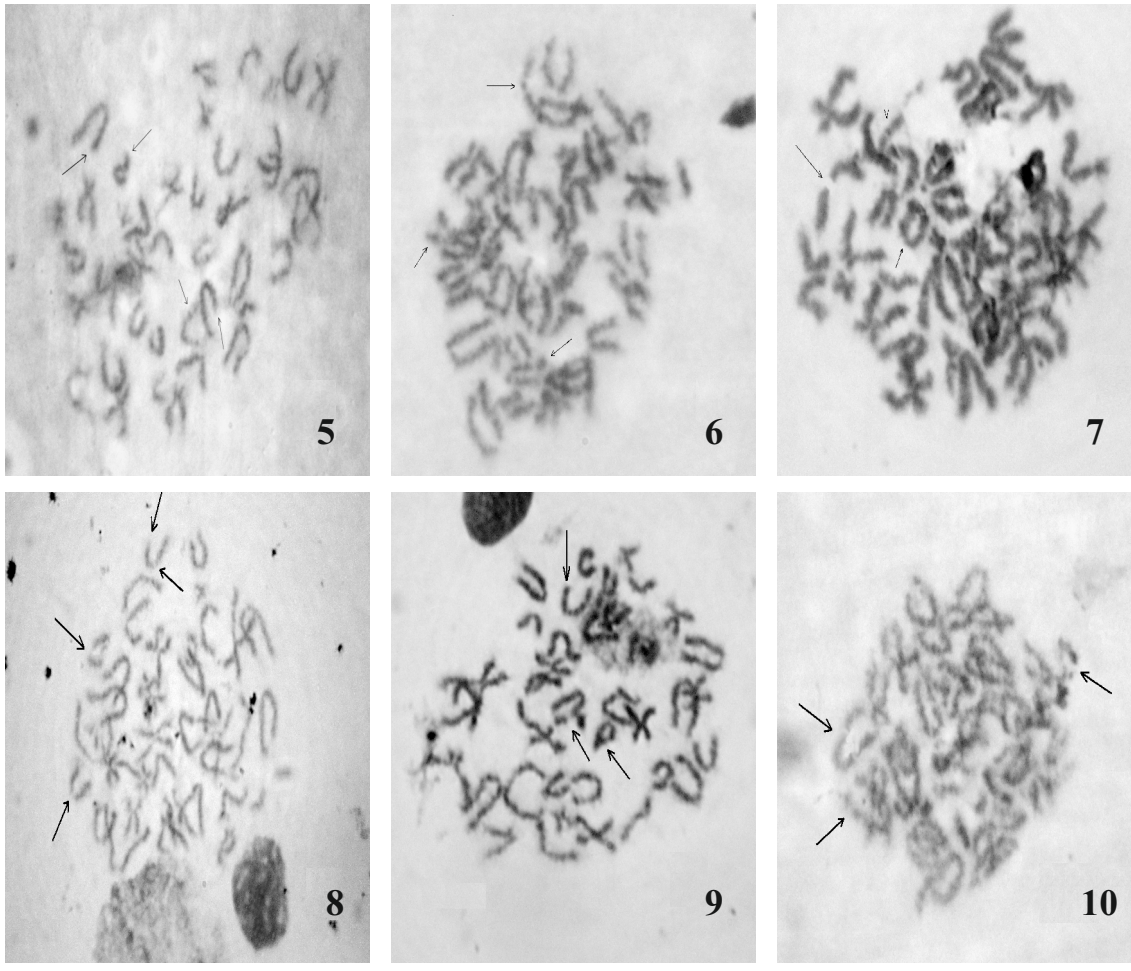
Treated series

In this series, 7 types of structural chromosomal aberrations were observed (Figs 8–10).The maximum had been scored with centromeric dissociation (2.90%) and minimum with ring chromosome (0.70%) (Table 3, Fig. 3). In terms of time intervals, the highest aberration was obtained after 16 h (12.38%) and lowest was after 1 wk (5.76%) post exposure (Table 3, Fig. 4).

The χ^2 value for this treated series are, 36.24 for iso-chromatid gap, 10.46 for centromeric dissociation, 28.08 for iso-chromatid break, 18.68 for translocations. The overall χ^2 value is 29.33 for the pooled data. All these values are highly significant. The other values are less significant. The overall F-value is 42.31 which is significant at 1% level – highly significant (Table 5).

DISCUSSION

The first report on somatic chromosome



Figs 5–10: 5–7. Photomicrographs showing various chromosomal aberrations induced by 0.8 Gy gamma rays. 5. Chromatid break, sub-chromatid gap, translocation (arrows). 6. Iso-chromatid gap, chromatid break (arrows). 7. Ring chromosome, centromeric dissociation, iso-chromatid break (arrows). 8–10. Photomicrographs showing various chromosomal aberrations induced by 2.4 Gy gamma rays 8. Chromatid break, centromeric dissociation, sub-chromatid gap. 9. Chromatid break, ring chromosome. 10. Translocation, iso-chromatid break, centromeric dissociation.

number of *S. murinus* seems to have been cited by Borgaonkar (1969). The diploid number 40 was subsequently confirmed by Manna & Talukdar (1967) and Ray-Chaudhuri et al. (1968) for the Indian taxon. The chromosome map of *Suncus* was reported by Kuroiwa et al. (2001). The researchers used direct R-banding fluorescence in situ hybridization (FISH) method to map 18S-28S ribosomal RNA genes and 10 human cDNA clones on the chromosomes of the musk shrew (*S murinus*). The comparative mapping of 10 cDNA clones of human chromosome 1 demonstrated that human chromosome 1 consisted of at least 3 segments homologous to *Suncus* chromosomes

(chromosomes 7, 10, and 14). Earlier; Sur et al. (2012a, 2012b) assessed the effect of 2 different doses of X rays (80r and 120r) on bone marrow cells of mice, *Mus musculus*. Moreover, Das et al. (2011) further investigated the effect of 2 different doses of γ rays (80 r and 240 r) on bone marrow cells of laboratory mice. Earlier it was observed that the dose 240 r γ rays is more genotoxic to mice chromosomes than 80 r, the aberrations are non-random in distribution, they are somewhat time-dependent and centromeric regions of the chromosomes are most vulnerable to γ irradiation. Same observations are reported in the present study. But 240 r dose of γ rays was also observed to induce sub-lethal physiological changes as accumulation of peritoneal fluid in stomach and tumour formation near the nose region in the mice. But such events did not occur in the shrews of the present study.

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RESEARCH ARTICLE

CYTOLOGICAL STUDIES ON TWO SPECIES OF APHIDS INFESTING MEDICINAL PLANTS FROM KANGRA DISTRICT OF HIMACHAL PRADESH

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SUMMARY Chromosomes of 2 species of aphids, infesting different host plants were studied from Kangra district of Himachal Pradesh. These species are, *Aphis gossypii* infesting host plant *Hibiscus rosa-sinensis,* and *Macrosiphoniella sanborni* infesting *Chrysanthemum indicum.* The diploid chromosome number of *A. gossypii* is 8 and *M. sanborni* is 12. The actual lengths as well as relative lengths of chromosomes of somatic metaphase plates and the total complement length were calculated for both the species and their karyotypes were prepared. On the basis of relative length data idiograms were constructed.

Keywords: Chromosomes, holocentric, aphids, total complement length, karyotype, idiogram.

INTRODUCTION

Aphids are tiny phloem feeders and soft bodied insect pests that have worldwide distribution, with most of the species occurring in temperate regions where approximately 1 in 4 plant species is colonized by at least 1 species of aphids (Dixon 1998). There are about 5000 described aphid species worldwide out of which 510 presently accepted genera infesting about 300 plant families (Blackman & Eastop 2000, 2006, 2015, Favret 2015, Gavrilov-Zimin et al. 2015) and among 510 species, chromosomes of only 24% species have been reported (Gavrilov-Zimin et al. 2015). However, only 250 species of aphids, out of the entire rich aphid fauna are currently regarded as economically significant pests (Blackman & Eastop 2006, Van Emden & Harrington 2007).

According to Heinrich (2000, 2003) the modern medicines are developed in 20th century on the basis of traditional uses of the plants. According to Ngo et al. (2013) the secondary metabolites preserved in stem, leaves, fruits and bark of plants are reliable, accessible, safe and inexpensive for the treatment of many diseases. The host plants of present study, *Hibiscus rosa-sinensis* and *Chrysanthemum indicum* have great potential to cure multiple diseases and aphid pests are damaging these plants and also reducing most of their medicinal properties. During the field examination of present study, *A. gossypii* and *M. sanborni* were found prominently in large numbers on these host plants.

There are many features in aphid's life cycle like viviparity, polymorphism, holocentric chromosomes, host alternation and telescoping of

generation which makes it complex (Gautam et al. 1993). From India, Sethi & Nagaich (1972) reported chromosomes of *Myzus persicae* having 2n = 12 and 14 in different clones. Later, several workers reported chromosomes of aphids from Himachal Pradesh (Gautam & Verma 1982, Gautam & Sharma 1990, Dutta & Gautam 1993). Gautam & Kumari (2003) reported karyotype of green apple aphid, *Aphis pomi* from India. Samkaria et al. (2010) reported karyotypes of some commonly occurring aphid species in which 2n ranged from 8 to 18. Guleria & Gautam (2015) reported karyotypes of 7 species of aphids from Mandi district of Himachal Pradesh. Khagta & Gautam (2016) studied chromosomes of 6 species of aphids with diploid chromosome number ranging from 2n = 8 to 12. Recently, some other workers reported chromosome numbers of aphids ranging between 2n = 4 and 22 (Sharma & Gautam 2019, Kumari et al. 2022, Anupriya & Kumari 2021, Singh et al. 2021).

The largest aphid genus *Aphis* comprises more than 500 species (Eastop & Hille Ris Lambers 1976). *A. gossypii* is a major pest of cotton and cucurbits, and in glasshouses in cold temperate regions (Blackman & Eastop 2000). *A. gossypii* is an important vector for viral diseases and can transmit more than 50 different viruses, including cucumber mosaic virus. It extracts nutrients and disturbs balance of growth hormones. Genus *Macrosiphoniella* comprises of about 115 Old World and 5 North American species. At least half the species feed on *Artemisia* spp. and a few on *Chrysanthemum* spp. (Blackman & Eastop, 1984). Chrysanthemums are prone to a large number of viral diseases including mosaic, chrysanthemum smut virus, which are spread by *M. sanborni* aphids. These viral diseases cause spindly stunted shoots, yellowed mottled foliage, which affect the growth and quality of plants. So, it is worthwhile to investigate the cytology of these aphid species.

Chromosomal polymorphism occurs in aphids as a result of fusion or dissociation of chromosomes which leads to evolution of new biotypes. So, it is very important to study the chromosomes of aphids from different plants of different geographical regions. Kangra is rich in vegetation where many sucking insect pests like aphids cause a lot of damage. There is very little information on aphids and their chromosomes available from this area. Keeping this in view, it was considered desirable to investigate the cytology of aphids from this area. This study will help in understanding the host-aphid association to each other and taxonomy of aphid species. Karyotypic study on *A. gossypii* and *M. sanborni* would be useful in understanding different cytological mechanisms involved in their life cycle and evolution of new biotypes.

MATERIALS AND METHODS

Apterous, parthenogenetic, viviparous female aphids infesting twigs, leaves and inflorescence of *H. rosa-sinensis* and *C. indicum* were collected from Ansoli and Matour area of Kangra. Multiple morphs of *A. gossypii* were found from dark green to pale yellow. These are collected from stem, leaves, petioles and floral buds of *H. rosa-sinensis*. *M. sanborni* were dark reddish brown colour and were collected from young apical shoots and stem of *C. indicum.* For identification of aphid species key developed by Blackman & Eastop (2000) was used.

For chromosome studies, somatic embryonic

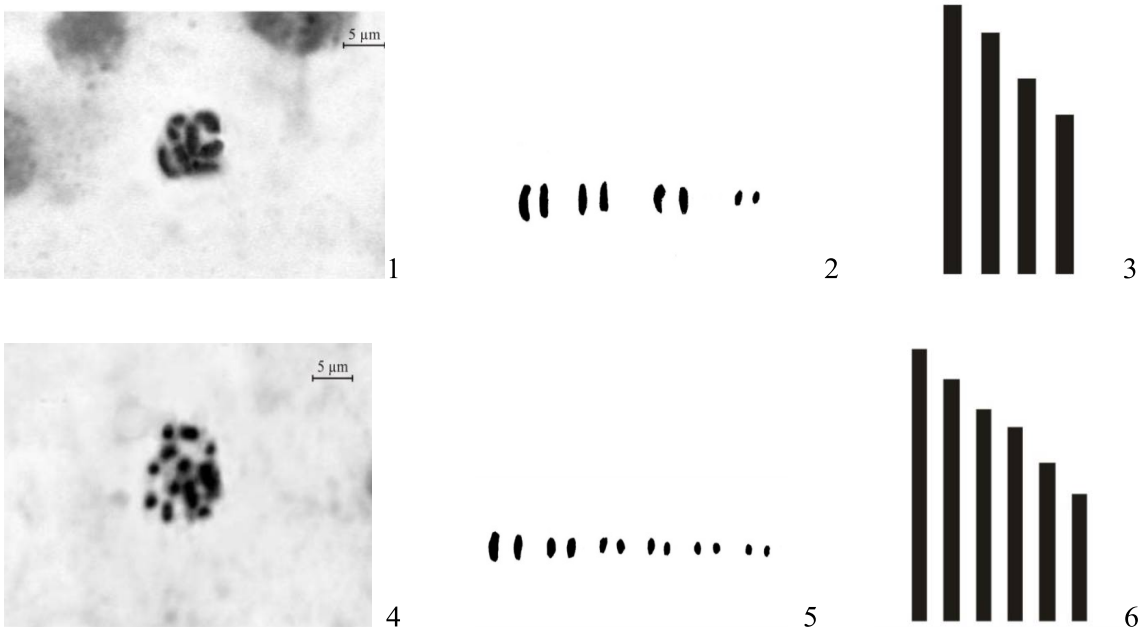
tissue of parthenogenetic females were used. They were dissected by puncturing the posterior end of abdomen for embryos and pretreated with 0.8% tri-sodium citrate solution for 25–30 min. These embryos were fixed in 1:3 acetic-methanol solution for 15–20 min. After fixation, embryos were put on glass slide in a drop of 45% acetic acid for 3–5 min. A coverslip was put on the material with one edge extended outside the slide. The slide along with coverslip was then placed between 2 layers of blotting papers and tapped gently with the blunt end of the forceps. Coverslip was dislodged off the slide with a sudden jerk. Both slides and coverslips were then dried in a dust free chamber and stained in 2% Giemsa for 20–30 min. Slides were made permanent by dipping in xylene and mounting in DPX.

The slides were observed under a binocular microscope and photomicrographs were taken. The actual lengths of chromosomes were measured using ocular micrometer. From the actual lengths of chromosomes, total complement lengths and relative lengths were calculated. The idiogram for each species was constructed based on relative length data.

OBSERVATIONS

A. gossypii Glover

This species has diploid chromosome number of 8 (Figs 1, 2). The chromosomes are holocentric. The actual length of chromosomes ranged from 1.96 µm ± 0.05 S.E. to 3.32 µm ± 0.05 S.E. Total complement length was 21.35 µm ± 0.22 S.E. Relative length of chromosomes ranged from 9.18 ± 0.25 S.E. to 15.58 ± 0.29 S.E. The idiogram



Figs 1– 6: Karyotypes of aphids. 1–3. *A. gossypii.* 1. Somatic chromosomes. 2. Karyotype. 3. Idiogram. 4–6. *M. sanborni*. 4. Somatic chromosomes. 5. Karyotype. 6. Idiogram. (Scale = 5 µm)

of this species is given in Fig. 3, showing a pair of long chromosomes, 2 pairs of medium size and 1 pair of short chromosomes.

***M. sanborni* (Gillete)**

The diploid chromosome number in this species is 12 (Figs 4, 5). The chromosomes are holocentric. The length of chromosomes ranged from 0.45 µm ± 0.05 to 2.69 µm ± 0.05. The total complement length was 17.60 µm ± 0.42. The relative length of chromosomes ranged from 2.55 ± 0.23 to 15.36 ± 0.49. The idiogram showed a pair of long chromosomes, 2 pairs of medium size and 3 pairs of short chromosomes (Fig. 6).

DISCUSSION

A. gossypii has diploid chromosome number of 8. Earlier, many workers have also reported the same chromosome number for this species (Robinson & Chen 1969, Gut 1976, Kurl 1978, Kulkarni & Kacker 1979, Stevens 1906, Dutta & Gautam 1993, Gautam & Dhatwalia 2003, Devi & Gautam 2012, Guleria & Gautam 2015). Lal & Kurl (2016) reported 2n = 8 and 9 for *A. gossypii* as reported earlier by Anupriya & Gautam (2017).

M. sanborni with 2n = 12 reported here is in conformity with the earlier reports of Blackman & Eastop (2015), Boschetti (1963), Dutta & Gautam (1993), Gautam & Sharma (1990). Earlier, Chen & Zheng (1985) reported diploid chromosome number of 10 in *M. sanborni*. Samkaria et al. (2010) and Sharma & Gautam (2019) studied the karyotype of *M. sanborni* and reported 2n = 12.

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RESEARCH ARTICLE

IN-SILICO PROMOTER ANALYSIS OF *MSP1* GENE CO-EXPRESSING NETWORK IN RICE

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SUMMARY The rice *MULTIPLE SPOROCYTE1* (*MSP1*) gene, which encodes a leucine-rich repeat receptor-like kinase, is crucial for efficient rice reproduction because it exerts feedback inhibition from the megasporocyte, preventing neighbouring cells from accessing the germ line. This study aims to understand the regulation of *MSP1* promoter and other comparable promoters. The co-expression network of *MSP1* gene were generated, and the tandem repeats, the transcription factor binding sites (TFBS), and novel potential *cis*-motifs present in the promoter regions of the 50 co-expressed genes were predicted. These promoters comprised 7992 potential TFBSs representing 7 transcription factor (TF) families. The most common TFBS for these promoters belonged to the family of 'TEOSINTE BRANCHED1 (TBI1), CYCLOIDEA (CYC), and PROLIFERATING CELL NUCLEAR ANTIGEN FACTORS (PCF1 and PCF2)' proteins, abbreviated as TCP, which is involved in flowering and gametophyte development. Three novel motifs were predicted, of which, 2 indicated a connection with ethylene responses, which is crucial for gametophyte development. Thus, this study gives an insight into the possible role of *MSP1* and its co-expressed promoters in gametophyte development.

Keywords: Promoter, *Oryza sativa*, transcription factor binding site, gametophyte, *MSP1*.

INTRODUCTION

The success of plant reproduction is influenced by a number of biochemical processes including cell signaling and controlled division of certain cells. To get a deeper insight into the biology of plant reproduction, a variety of molecular, biochemical, physiological and environmental factors influencing reproductive success must be investigated. A vital step towards this is the analysis of gene networks that exclusively regulate gametophyte development. An in-depth examination of haploid plant gametophytes is essential to characterize these genes involved in reproduction. A plethora of genes considered to be necessary for the generation and regulation of haploid gametophytes have been previously characterized, mostly in maize (Vollbrecht & Hake 1995) and *Arabidopsis* (Grossniklaus & Schneitz 1998) by analyzing chromosomal and T-DNA-insertion mutants respectively.

In order to characterize a gene and study its function, the elucidation of its promoter, identification of the conserved motifs, and understanding its transcriptional regulation, is essential.

Transcription factors (TFs) regulate transcription by binding to certain DNA sequences in the promoter (Latchman 1997). Gene expression is controlled by the activation or repression of TFs, which are required for a variety of important cellular functions. It is challenging to find most of the *cis*-regulatory motifs with current tools since many TFs remain unexplored. Therefore, predicting genes and their regulation based on TF binding sites (TFBS) in the promoter region is a major topic in system biology.

Rice (*Oryza sativa*) is in cultivation for over 9000 y, and it feeds more than half of the world's population. Rice production will encounter significant challenges in the coming decades due to its high water and manpower requirements. Understanding more about the genes involved in gametophyte development and reproduction would aid scientists in mitigating these obstacles through molecular breeding methods. Thus, understanding the promoter regulation of gametophyte-specific genes is important. *MSP1* is a rice gene reported to be important for regulating plant reproduction. *MSP1* encodes a protein kinase with a Leu-rich repeat (Nonomura et al. 2003). The retrotransposon mutation of *MSP1* resulted in an overabundance of both male and female sporocytes, disorganised female gametophytes, disruption of anther wall and tapetum layers, and lack of development of pollen mother cells (Nonomura et al. 2003). *MSP1* and OsTDL 1A make a functional complex that start the construction of the anther wall and regulate the number of cells that can undergo male and female sporogenesis (Zhao et al. 2008). Similarly, the *MSP1* orthologue *EMS1* in *Arabidopsis* also shows male sterility in mutants (Zhao et al. 2008). This study is aimed to identify the *MSP1*-co-expressed genes, analyse their TFBSs, and identify tandem repeats and novel motifs present in their promoters. This basic information provides foundational knowledge to compare *MSP1* and its co-expressed genes that can be compared in other plants to promote successful reproduction and hybrid plant development of rice.

MATERIAL AND METHODS

All computing tasks were performed using the Windows operating system. The sequence accession number of rice *MSP1* cDNA was obtained from Nonomura et al. (2003). The PlantCare web tool, was used to evaluate the full sequence of the *MSP1* promoter and the distribution of motifs (Lescot et al. 2002)**.** The co-expression network of *MSP1* was generated with the *Arabidopsis thaliana* trans-factor and *cis*-element prediction database (ATTED II) tool (Obayashi et al. 2018). The Chromosome Map Tool, accessible from *Oryza* Base (http://viewer.shigen.info/oryzavw/maptool/MapTool.do) was used to create the chromosome maps of the genes that co-express with *MSP1*. The Plant Promoter Database (PPDB) (Hieno et al. 2014) was used to extract the promoter sequences of *MSP1*-co-expressed genes. The regions one kb upstream of the ATG start codon of genes were downloaded and divided into 2 segments namely, the proximal promoter (+1 bp to -500 bp) and the distal promoter (-501 bp to -1000 bp) for further analysis. Putative TF binding sites (PTFBS) were identified using the PlantPAN 2.0 tool (Chow et al. 2016). The 'Multiple promoter analysis' method was applied under gene group analysis, and the presence of the same TFBSs in distinct promoter sites was discovered. Species were set to '*Oryza sativa*'. The rest of the conditions were

kept as default.

The analysis of short tandem repeats in the promoter regions was performed by Tandem Repeats Finder (TRFi) with default settings. One kilo base upstream sequences flanking the ATG start codon collected from the PPDB were subjected to the command line application of 'Multiple Expectation Maximisations for Motif Elicitation' (MEME) Suite (v5.0.5) for de novo *cis*-element prediction, overrepresented in the gene network. This algorithm was used to look for motifs with lengths of six to 50 nucleotides and E values less than one. The 'zero or one occurrence per sequence' (zoops) option was used to conduct motif searching for each set of upstream regions. TOMTOM web programme were utilized to compare predicted motifs with known moifs reported.

OBSERVATIONS

Fifty top co-expressed genes of the *MSP1* (Entrez ID: 4324355) gene were identified using the ATTED 2 tool (Table 1). Four genes with Entrez IDs, 9267373, 107280430, 4329691 and 4332820 showed direct connections with the *MSP1* gene in the co-expression network (Fig. 1). These 4 genes were in turn connected to 46 other genes. Chromosomal positions of the 51 co-expressed genes were located *in silico* through *Oryza* Base (Fig. 2). Chromosome 2 had the maximum number of 10 co-expressed genes on them. None of them was located on chromosome 12. The least number of genes (1) was found on chromo-somes 6, 7 and 8. Some genes were located in relatively closer proximity, as groups of 4–7 linked genes (Fig. 2).

The number of PTFBS of 7 TF families, basic leucine zipper (bZIP), SQUAMOSA promoter-binding-protein (SBP), basic helix-loop-helix (bHLH), Homeodomain TALE, B3, NF-YB and TCP were identified in the promoters of *MSP1* and its 50 co-expressed genes. The total number of PTFBS varied for each category (Fig. 4). While TCP had the highest number of 3948 sites, SBP had the lowest number of 190 sites (Fig. 4). Individual gene-wise analysis conveyed that not all genes had the PTFBS for all the 7 families. For example, the putative binding sites (PBS) for bZIP were absent in the promoters of 4 genes, *LOC4332017*, *LOC4331247*, *LOC-4328423* and *LOC4328122*, encoding Protein S-acyl-transferase 22, Protein furry, Cullin-associated NEDD8-dissociated protein 1 and a Conserved hypothetical protein respectively. While the promoter of *LOC4332820* (an uncha- racterized gene) had 20 PBSs for the bZIP family, *MSP1* promoter had only one PBS for bZIP. Distribution study on the positive and negative strands revealed that the PTFBS were more or less similarly distributed across both the strands of most promoters (Fig. 3). Similarly, most of these TFs had random distribution of their PBSs across the proximal and distal parts of the promoter.

Tandem repeats of length ranging from 2 to 63 bp were seen in the promoters of 12 genes (23.53%), including *MSP1* (Table 2) . While most promoters had one type of repeat, one promoter, *LOC4329033* had 3 types of repeats, and 2 promoters, *LOC4325878* and *LOC4330278* had 2 types of repeats. Hence, a total of 16 types of repeats were found in the promoters of 12 genes. Most of these repeats were AT-rich, with the highest GC content of 27.27% in one gene, *LOC4324355*. In all remaining repeats, GC content was extremely low, in the range of 0–0.67%.

Three potential motifs, motif 1 (MT1), motif 2 (MT2), and motif 3 (MT3) of lengths 41, 21 and

TABLE 1: Details of *MSP1* co-expressing genes and their known functions.

Sl. No.	Gene name	Gene symbol	Function (based on ATTED II)	Entrez gene ID
1	UK	*LOC4332820*	Uncharacterized	4332820
2	UK	*LOC107280430*	Protein TORNADO 1-like	107280430
3	UK	*LOC4329691*	Uncharacterized	4329691
4	UK	*LOC4350800*	Uncharacterized	4350800
5	*D6PKL1*	*LOC4336568*	Serine/Threonine-protein kinase D6PKL1	4336568
6	UK	*LOC4332017*	Probable protein S-acyltransferase 22	4332017
7	UK	*LOC4331748*	F-box/kelch-repeat protein At1g55270	4331748
8	*RLT2*	*LOC4325898*	homeobox-DDT domain protein RLT2	4325898
9	UK	*LOC4337567*	PH: RCC1 and FYVE domains-containing protein 1	4337567
10	UK	*LOC9271054*	Uncharacterized	9271054
11	UK	*LOC4346704*	Uncharacterized	4346704
12	UK	*LOC4345949*	Uncharacterized	4345949
13	UK	*LOC4326767*	2-oxoglutarate-Fe(II) type oxidoreductase hxnY	4326767
14	UK	*LOC4338654*	PH: RCC1 and FYVE domains-containing protein 1	4338654
15	*LOL2*	*LOC4325320*	Fibrous sheath CABYR-binding protein	4325320
16	UK	*LOC4331573*	Uncharacterized	4331573
17	UK	*LOC4329656*	ACT domain-containing protein ACR3	4329656
18	*ASP1*	*LOC4344728*	Protein TPR2-like	4344728
19	UK	*LOC4340705*	Carbon catabolite repressor protein 4 homolog 3	4340705
20	UK	*LOC9267456*	Inactive protein kinase SELMODRAFT_444075	9267456
21	UK	*LOC4336125*	Uncharacterized	4336125
22	UK	*LOC4331247*	Protein furry	4331247
23	*ELF1*	*LOC4332159*	U-box domain-containing protein 75-like	4332159
24	UK	*LOC9267373*	Uncharacterized	9267373
25	UK	*LOC4334563*	Uncharacterized	4334563
26	UK	*LOC4328423*	Cullin-associated NEDD8-dissociated protein 1	4328423
27	UK	*LOC9268504*	Uncharacterized	9268504
28	UK	*LOC4336196*	Programmed cell death protein 4	4336196
29	UK	*LOC4328539*	DNA repair protein RAD50	4328539
30	UK	*LOC4349866*	Uncharacterized	4349866
31	*EMF1*	*LOC4325878*	Protein EMBRYONIC FLOWER 1	4325878
32	UK	*LOC4331505*	Bromodomain-containing protein DDB_G0270170	4331505
33	UK	*LOC4328122*	Uncharacterized	4328122
34	UK	*LOC4336647*	Mitogen-activated protein kinase kinase kinase YODA	4336647
35	UK	*LOC4334928*	SWI/SNF complex subunit SWI3D	4334928
36	UK	*LOC4349254*	Uncharacterized	4349254
37	UK	*LOC4330691*	Uncharacterized	4330691
38	*SCAMP6*	*LOC4336849*	Secretory carrier-associated membrane protein 6-like	4336849
39	UK	*LOC4349037*	Pentatricopeptide repeat-containing protein At2g20710: mitochondrial	4349037
40	UK	*LOC4330278*	Uncharacterized	4330278
41	UK	*LOC4329033*	Protein FAR1-RELATED SEQUENCE 5	4329033
42	UK	*LOC4336751*	Uncharacterized LOC4336751	4336751
43	UK	*LOC4326327*	Uncharacterized	4326327
44	UK	*LOC4325443*	Uncharacterized	4325443
45	UK	*LOC4339386*	Anthocyanidin 5:3-O-glucosyltransferase	4339386
46	*FTIP7*	*LOC4338582*	FT-interacting protein 1	4338582
47	UK	*LOC4343449*	Uncharacterized	4343449
48	UK	*LOC4349020*	Protein STABILIZED1	4349020
49	UK	*LOC4325575*	Alpha-amylase 3: chloroplastic	4325575
50	UK	*LOC4328584*	Methyl-CpG-binding domain-containing protein 9	4328584

UK, Names of these genes are unknown.

TABLE 2: List of *MSP1* co-expressing genes with tandem repeats in *O. sativa* promoter regions.

Sl. No.	Gene symbol	Period size	Copy No.	Repeat sequence	GC %	AT %
1	*LOC4324355*	33	1.9	AACTCTAACGAATTCTTTGCGTCAA AAAAAAAA	27.27	72.73
2	*LOC4332820*	3	9.0	ACC	0.67	33.33
3	*LOC4350800*	36	2.0	TTATTGGTGCTATTAGATTGGATCAT GACGCCAATG	0.39	61.11
4	*LOC4331748*	38	2.0	TTTTTTATATTTACATAAAATTTTTA AATAAGACGAGT	0.13	86.84
5	*LOC4338654*	20	2.3	AAAAAAAAAAGGGGAGAGGA	0.35	65
6	*LOC4340705*	19	2.5	AAATTTTGGCAAAGTTGCCA	0.37	63.16
7	*LOC4334563*	2	22.5	AT	0	100
8	*LOC9268504*	18	2.3	AAGTTTATG-TGTG-TAGAA	0	100
9	*LOC4325878*	2	14.5	TC	0.50	50
		7	3.9	TTTGTGG	0.43	57.14
10	*LOC4330278*	44	2.0	TTGATCCTTACTCTGAACCTATCCTA ATTTAGAGAACTGATTTA	0.32	68.18
		39	2.1	GCAATTCACTTGGGCACATAATGAT GCGTGATTGGTTGG	0.46	53.85
		21	9.6	CGTATAGAAAGTTTGTATACG	0.33	66.67
11	*LOC4329033*	63	3.2	GTATAGAAAGTTTGTATACGCGTAT ATAAACTTTGTATACACGTATAAAA AGTTTGTATACAT	0.25	74.60
		23	2.1	TACGAATACAAACTTGGTATATA	0.26	73.91
12	*LOC4339386*	30	2.1	TACTAACATGTGGGTCCCACTTTTTT TTTT	0.33	66.67

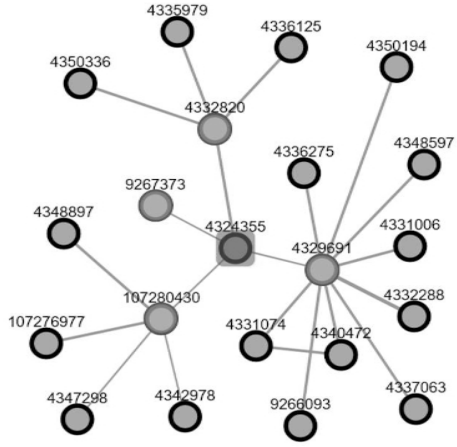


Fig. 1: Co-expression network of *MSP1* gene generated using ATTED II tool. Numbers in the image indicate the Entrez ID of genes. The gene, *4324355*, is the *MSP1* showing direct links with 4 genes, *4332820, 9267373, 107280430* and *4329691*.

29 nucleotides respectively, were predicted using the MEME Suite software, from the promoters of 51 co-expressed genes (Figs 5–7). These 3 motifs repeated 51, 51 and 29 times respectively, in these promoters (Fig. 9). While MT1 and MT2 were located in all 51 promoters, MT3 was least prevalent, appearing in just 28 of 51 (54.9%) promoters. The distribution of motifs was much greater in the positive strands than in the negative strands (Fig. 8). Motif concentrations were highest (67.17%) between -500 and +1 bp (proximal promoter) upstream of the translation start site. The 3 newly found motifs were then used as a query motif for comparison against a database of known motifs in *O. sativa* using the

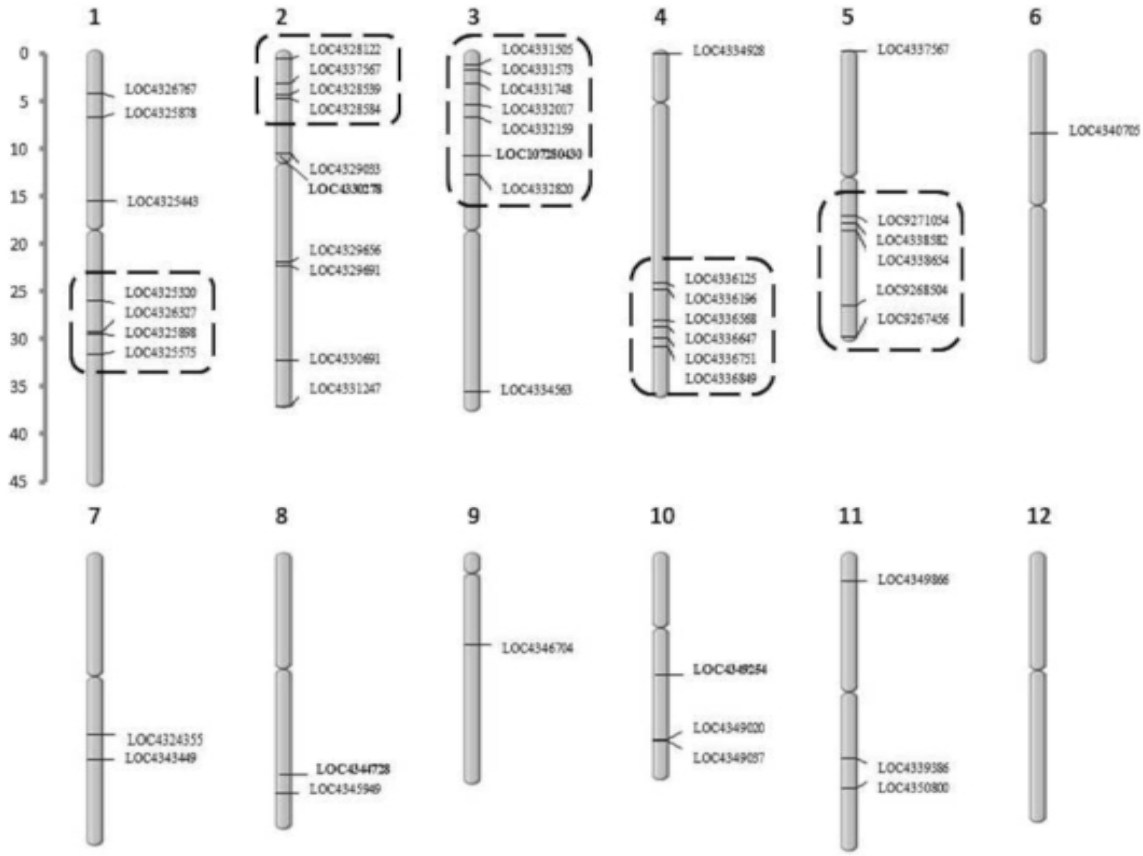
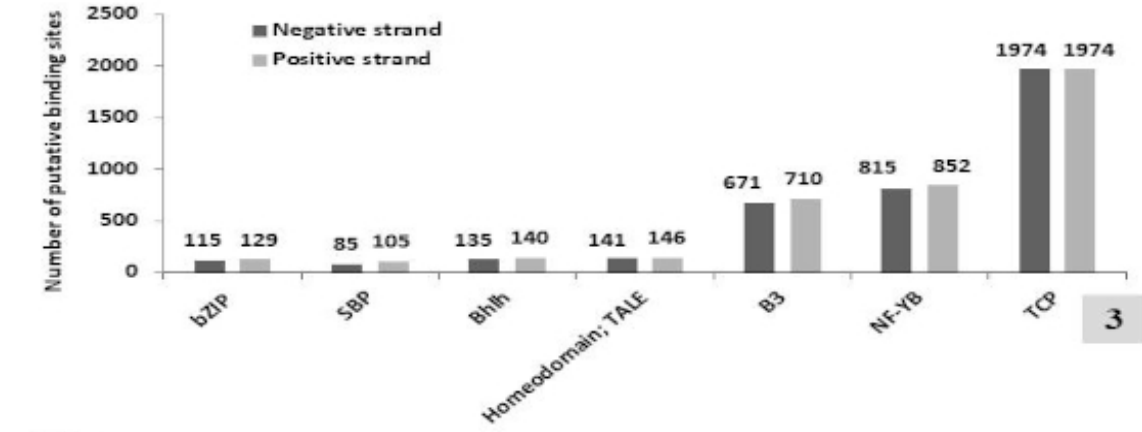


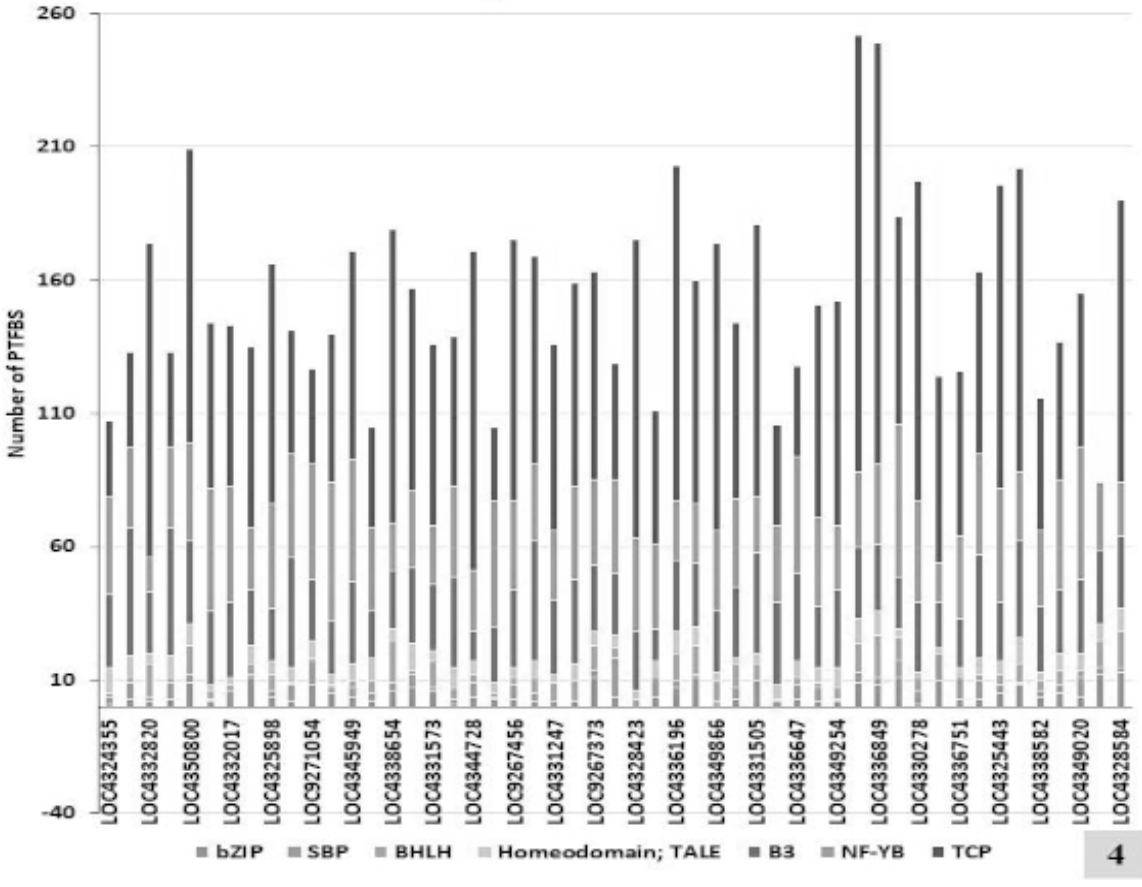
Fig. 2: Chromosomal distribution of rice *MSP1* and its 50 co-expressing genes generated using *Oryza* Base tool. The numbers above each chromosome indicates the respective chromosome numbers. The locations of the 51 genes are marked along with their respective accession numbers. The dotted lines indicate clusters of linked genes. The scale represents length in mega base pairs.

TOMTOM web programme. Out of 366 known TF motifs in the database, MT1, MT2, and MT3 matched with 38, 28, and 43 motifs respectively. The best suggested candidate TF for binding with MT1 was MO1602 (OS09G0572000), MT2 was MO6863 (OS07G0236700), and MT3 was MO6670 (OS05G0497300). The MT1 and MT3 potential targets, MO1602 and MO6670 belong to the AP2 transcription factor family, whereas the MT2 target, MO6863 belongs to Dof family.

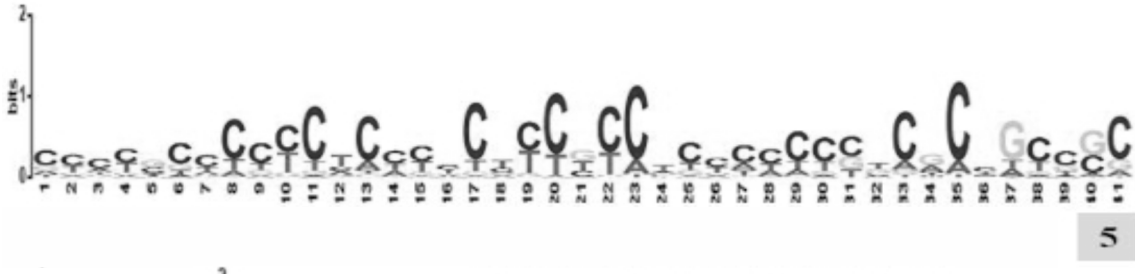
DISCUSSION

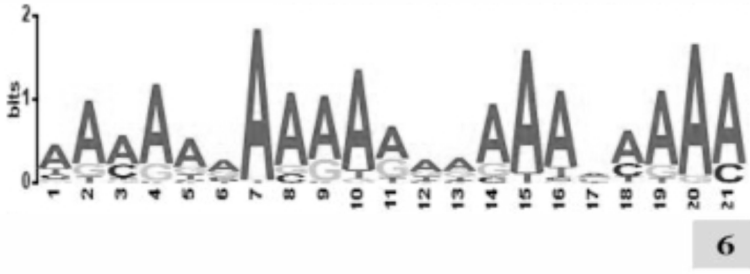
Genes with similar functions frequently share characteristics, such as genetic or physical interactions, as conveyed by the 'Guilt-by-association' theory (Oliver 2000). Following this paradigm, co-expression analysis has become an effective method for developing ideas regarding gene function in the last decade (Wolfe et al. 2005). The idea is that the genes with similar expression in same tissues or in response to different challenges could be associated with an identical cellular mechanism. Genes included in the co-expression network can be used to characterise promoters, their regulatory features, and to find out novel *cis*-elements in them. In this

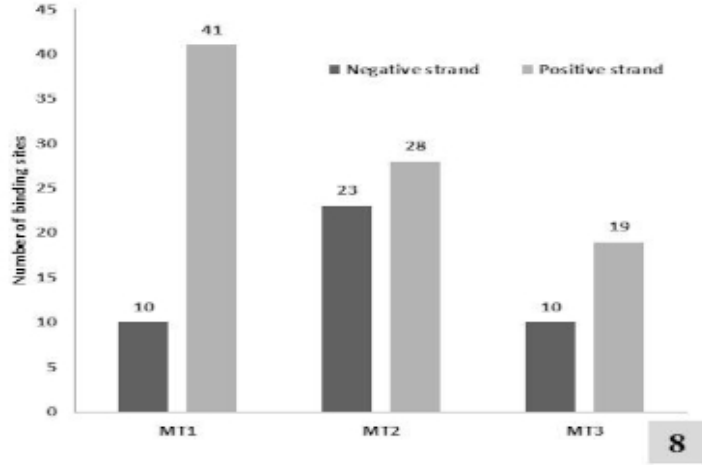




Figs 3 & 4: Putative transcription factor binding sites (PTFBS) present in *MSP1*-co-expressed gene promoters. 3. Total number of PTFBS identified on the negative and positive strands of co-expressing promoters. 4. Diversity of PTFBS distribution on various gene promoters.







Figs 5–**8:** Novel motifs in the promoters of 50 genes that co-expressed with *MSP1*. 5. Conserved putative motif 1 (MT1). 6. Conserved putative motif 2 (MT2). 7. Conserved putative motif 3 (MT3). X axis in the a, b and c is the number of nucleotides, indicating the length, Y axis indicating the bits (relative positional frequency of a particular nucleotide). 8. Total number of novel motifs identified on the negative and positive strands of co-expressing promoters.

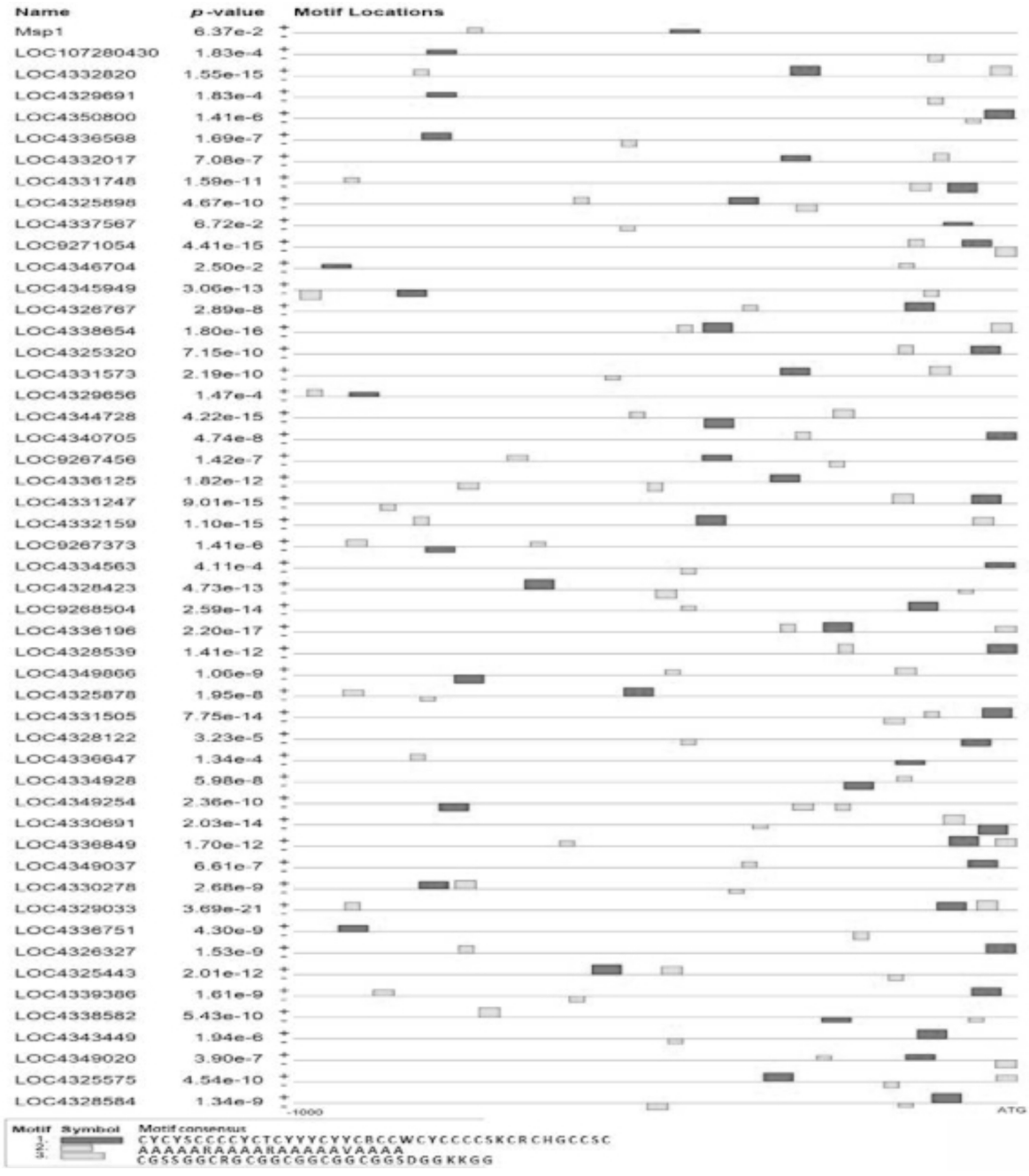


Fig. 9: Distribution pattern of novel motifs predicted from the promoters (1 kb upstream of ATG start codon) of *MSP1* and its top 50 co-expressed genes, using MEME suite. The presence of colored boxes on the upper and lower side of the lines, represent their presence on positive and negative strands respectively.

study, we generated the network of rice *MSP1* gene, comprising of top 50 co-expressed genes. *MSP1* being gametophyte-specific, the promoters of the genes in this network could be carrying regulatory features for this type of expression. Thus, in this study, we profiled and analysed various regulatory regions of 51 promoters, inclusive of *MSP1*. Among the 4 primary genes which show direct connections with *MSP1*, the function of only one gene, '*TORNADO 1-like*' (107280430), which is ubiquitously expressed in mature seeds, flowers, grains and roots, is known (Cnops et al. 2006). Similar to *MSP1*, all the co-expressing genes of it show different levels of expression in flower and floral tissues indicating its role in gametophyte and sporophyte transition.

The locations of these genes were present across various chromosomes (except 12), with numbers ranging from 1 to 10 on a single chromosome. The close proximity of linked genes indicates that these could be under similar control of transcription regulation (Michalak 2008). The transcription regulation could be due to similar nuclear organization (Harmon & Sedat 2005) or influence by neighbourhood transcription due to non-coding RNAs (Carninci 2008), histone acetylation (Ebisuya et al. 2008), shared enhancer elements or other unknown factors (Ribeiro et al. 2022). Deeper analysis on this aspect could identify the correlation between their locations and co-expression.

Among the 7992 potential TFBSs, TCP TFs had the widest distribution across the promoters of all genes. Originally discovered in the late 1990s, TCP TFs are a class of plant-specific proteins that are conserved throughout the plant kingdom (Viola & Gonzalez 2023). TCP is an abbreviation for the names of the founder genes that were identified from 3 different species: *TEOSINTE BRANCHED 1* (*TB1*) from maize (*Zea mays*), *CYCLOIDEA* (*CYC*) from snapdragon (*Antirrhinum majus*), and *PROLIFERATING CELL NUCLEAR ANTIGEN FACTORS 1* and *2* (*PCF1* and *PCF2*) from rice (*Oryza sativa*) (Viola & Gonzalez 2023).TCP transcription factors are involved in several growth processes across a variety of plant species, including rice. They directly impact development through the cell cycle and indirectly through their effects on plant hormone signalling and the circadian rhythm (Danisman 2016). In rice and sorghum, they control shoot branching and panicle formation (Francis et al. 2016, Manassero et al. 2013). TCP16 is involved in the early phases of pollen production in *Arabidopsis*. Early pollen formation was aborted as a result of RNA interference with the *Arabidopsis* gene encoding the putative transcription factor TCP16 (Takeda et al. 2006). Therefore, the abundance of TCP TFBs in promoter regions suggests that the co-expressed genes and their promoters may play a major role in the development of male gametophytes.

The second-highest prevalent *cis*-element on co-expressed gene promoters was the NF-YB TFBS. According to Li et al. (2016), NF-Ys are among the most important families of transcription factors (TFs) involved in flowering. Recent study has also provided information on regulation dynamics of NF-Ys in plant flower development via participating in complex formation with diverse proteins, including CONSTANS (CO/B-BOX PROTEIN1 BBX1), a master flowering regulator in plants (Gnesutta et al. 2017, Shim et al. 2017). The third commonest TFBS were for the B3 superfamily (B3s), which represent a class of large plant-specific transcription factors that

play diverse roles in plant growth and development processes, including flowering induction (Ruan et al. 2021). It is also involved in the seed maturation process (Carbonero et al. 2017). High-level sequence conservation of the NF-Y TF family and presence of B3 TFs in the promoters suggests the possible role of *MSP1* and other 50 co-expressed genes in flower development.

The TALE gene family, also known as the Homeodomain; TALE gene family, is a significant transcription factor family that controls organ morphogenesis, signal transmission, and the development of meristems and fruits (Wang et al. 2022). Expression profiling of these genes during the course of ovule development in seeded and seedless cultivars suggested a potential role in ovule abortion associated with seedlessness (Li et al. 2017). The high presence of these TFBS is important in suggesting the role of co-expressed *MSP1* genes and promoters involved in female gametophyte development as well. In *Arabidopsis*, the meristem and inflorescence tissue growth is aided by the interaction of ATH1 (*Arabidopsis* homeobox 1) an TALE Homeodomain gene, STM (homeobox protein SHOOT MERISTEMLESS) and KNAT2 (*Arabidopsis* KNOX 2) (Wang et al. 2022).

bHLH domain-containing proteins, a common family of transcription factors in eukaryotes, are named for the distinctive basic helix-loop-helix region in their protein structures (Weber et al. 2014). A variety of plant developmental and metabolic processes, including photomorphogenesis, flowering induction, shade avoidance, and secondary metabolite biosynthesis, are controlled by bHLHs through transcriptional regulation of their target genes. These processes are crucial for promoting plant tolerance or adaptation to challenging environments (Guo et al. 2021). SBP-box family genes that are particular to plants are involved in controlling flowering time. The presence of SBP TFBSs on the proximal area of the GhD7 promoter validates *MSP1*'s involvement in determining flower development. The presence of bZIP TFBSs is significantly enriched in *MSP1* co-expressed gene promoters. The bZIP TFs have an established function of plants' responses to abiotic stress. The presence of an extensive number of bZIP *cis*-elements indicates that *MSP1* and its co-expressed genes also respond to abiotic stress in rice.

The eukaryotic genome comprises of many DNA repeats, which have an important function in genome evolution (Lopez-Flores & Gardo-Ramos 2012). This DNA may be scattered across the genome in tandem form or restricted to a single location. DNA tandem repeats are classified as microsatellites, minisatellites and megasatellites depending on the length of the repeated unit (Gemayel et al. 2010). Only 12 (23.53%) of the promoter regions of the co-expressed *MSP1* genes had tandem repeat units. Three of the 16 repetitions were microsatellites, while the other 13 were minisatellites. Megasatellites were absent among the promoters. The high AT content in the tandem repeats indicates that they are probably prone to more mutations because regions with high AT content mutate more frequently than those with high GC content (Fan & Chu 2007). In plants, short tandem repeats in promoters could be required for regulating gene expression in response to the dynamicity of the local environment (Reinar et al. 2021).

The identification of novel motifs residing in the promoter region of co-expressed genes is

considered a crucial step in studying the regulation of gene expression. Three novel motifs were predicted and named MT-1, MT-2, and MT-3 respectively. The proximal promoter region showed the presence of the majority of the novel motifs. This indicates that most of them might be part of the core promoters of linked genes. The transcription factors, MO1602 and MO6670, which are typically active in the ethylene-activated signalling pathway, are predicted to be able to recognise the motif MT-1 and MT-3. MT-2 is predicted to be recognized by MO6863 transcription factor which has a zinc finger DNA-binding domain that resembles to the Cys2 zinc finger (Kikuchi et al. 2003). Ethylene and auxin have been linked to the prevention of stamen formation (An et al. 2020). In *Arabidopsis*, stamen development stopped in ethylene-responsive *CsACO2* gene-overexpressed lines, suggesting that increased ethylene production limits stamen development (Duan et al. 2008). The results of several studies indicate that ethylene is essential for pollen dispersal. The 2 primary stages of anther dehiscence, which occur sequentially are, the degeneration of the intermediate layer, the tapetum, and the rupture of the anther wall at the location of the stomium between each anther's 2 locules (Wilson et al. 2011). It has been documented that the mutant melon ethylene receptor gene *Cm-ERS1/H70A* demonstrates a delay in tapetum programmed cell death, leading to the formation of aberrant pollen utilising transgenic tobacco plants (An et al. 2020). Several studies have examined the function of ethylene in the growth of female gametophytes, which eventually promotes ovule fertilization (De Martinis & Mariani 1999, Zhang & O'neill 1993). The ethylene biosynthesis gene, *ACO* is expressed prior to the development of the ovule in tobacco, and its suppression causes the development of the ovule to stop and prevents it from progressing to the stage of maturity. Therefore, ethylene-responsive transcription factor binding sites in the *MSP1* co-expressed gene promoters strongly indicate its crucial role in regulating male and female gametophyte development.

Thus, rice *MSP1* gene is considered an important gene that facilitates rice gametophyte development and successful reproduction. In the present study, we have developed the co-expressed genes of *MSP1* and analysed the promoter regions. Some of these genes were linked and located in close proximity in the chromosomes. TCP TFBSs were predominant among the 7 different TFBS families observed in the promoters of co-expressed genes. Most of the TFBS sites and their corresponding transcription factor families were observed to contribute mainly to flower development, plant development and gametophyte development. Two out of 3 novel motifs identified in the promoters were predicted to be involved in ethylene biosynthesis, which in turn could regulate gametophyte development. The findings of this study provide basic information on how the transcriptional regulation of *MSP1* and its co-expressed genes works in rice, which is involved in gametophyte development and plant reproduction. Therefore, this information, including the identification of novel motifs, might be used in developing synthetic promoters required for gametophyte-specific expression in rice, which have applications in breeding.

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RESEARCH ARTICLE

CHROMOSOMAL STUDIES ON SOME APHIDS INFESTING HORTICULTURAL CROPS OF SHIMLA HILLS

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SUMMARY This paper presents the cytological analyses of 3 aphid species, *Brachycaudus amygdalinus*, *Panaphis juglandis* and *Eriosoma lanigerum* infesting *Pyrus communis, Juglans regia* and *Malus domestica* respectively. *B. amygdalinus* and *E. lanigerum* have a diploid chromosome number of 12 and *P. juglandis* has 22. In all 3 species studied, the chromosomes are holocentric. Karyotype analyses were made and idiograms were constructed that showed a gradual decrease in the length of the chromosomes.

Keywords: Aphids, karyotype, chromosomes, holocentric.

INTRODUCTION

Aphids (Hemiptera), are small soft-bodied, phytophagous insects considered intrusive pests that are threatening the agricultural and horticulture ecosystem throughout the world (Blackman & Eastop 2006, Van Emden & Herrington 2007). About 653 species belonging to 208 genera of Aphididae are known from India (Agarwala & Gosh 1984). These are amongst the most devastating insect pests that exclusively feed on the phloem sieve tube elements of plants through their piercing-sucking mouth-parts (Tjallingii 1995). While probing, aphids inject their saliva inside the plant blocking the wound responses of plants by preventing the plugging of sieve tube elements and thus ensuring the continuous supply of phloem sap for hours from the single feeding site (Tjallingii 1995, Goggin 2007).

These are insects that have a high reproductive potential and different polymorphic forms that ensure their expansion and survival. They possess some peculiar features in their life cycle which include polyphenism, thelytoky, telescoping of generations, anholocyclic and holocyclic life cycle.

In addition, they are cytologically unique in having the holocentric chromosomes, where the spindle attachment is not localized so that, at anaphase, the chromatids do not move by a typical V-shaped figure, instead, they move apart in a parallel fashion (Hughes-Schrader & Schrader 1961).

In Himachal Pradesh, aphids are the major pests and severely damaging cash crops i.e. apple, pear, apricot, plum, etc. It is worthwhile to study aphid chromosomes to understand the different cytological mechanisms involved in their life cycle. These reinvestigations will be useful in determining the evolutionary trends in aphids and thus, may prove useful in ascertaining the

adaptability of these species to their host plants in different environmental conditions and geographic regions. The present study has been undertaken to analyse the karyotypes of three aphid species, *Brachycaudus amygdalinus, Panaphis juglandis* and *Eriosoma lanigerum.*

MATERIALS AND METHODS

Aphids were collected from the Kotkhai locality of the upper Shimla region of Himachal Pradesh, situated at an altitude range at 6800–6900 ft. above sea level. *B. amygdalinus* was collected from the newly forming shoots of the *Pyrus communis*, *P. juglandis* was collected from the young leaves of *Juglans regia* and *E. lanigerum* was collected from *Malus domestica*.

For cytological studies, young embryos (without an eye pigment) were selected, and standardized methodologies were employed in the laboratory in the following sequence. Firstly, pretreatment of the embryo in a 0.7 % trisodium citrate solution was done. Then, fixation was done in 1:3 glacial acetic acid and ethanol solution. After squashing in 45% acetic acid, Giemsa staining (2% Giemsa) was done followed by mounting in DPX. Permanent slides were observed using a binocular research microscope.

For identification of aphid species, the aphid mounts were prepared by boiling specimens in 95 % alcohol followed by boiling in a 10% KOH solution. Then, specimens were washed 2–4 times in distilled water. Dehydration was done in increasingly higher alcohol grades. Then, specimens were soaked in clove oil to clear them and mounting was done in DPX. Keys produced by Blackman & Eastop (1994, 2006) were followed for the identification of species.

OBSERVATIONS

B. amygdalinus

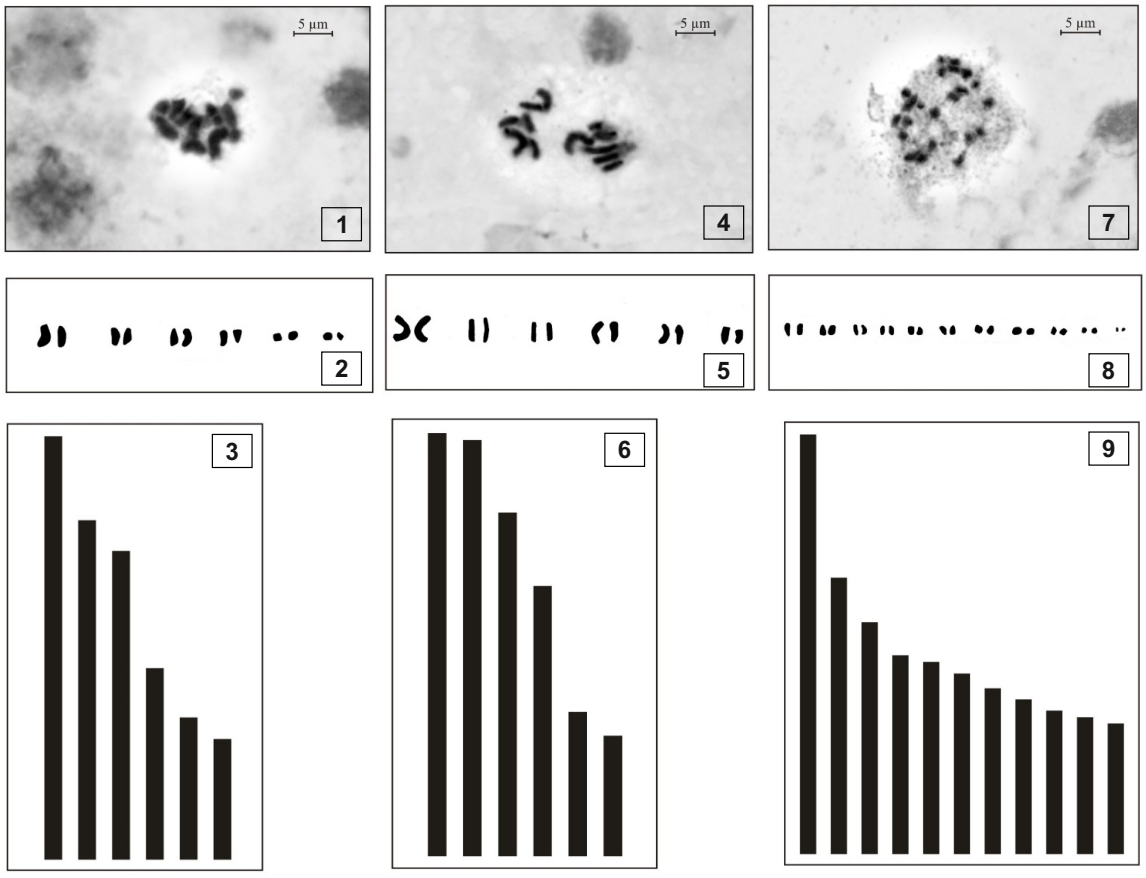
The diploid chromosome number in *B. amygdalinus* is 12 (Fig. 1). The chromosomes are holocentric. The measurements of chromosomes in 10 somatic metaphase plates showed that the actual mean length of the chromosomes ranged from 0.79 μm ± 0.014 S.E. to 2.82 μm ± 0.054 S.E. The mean total complement length was 20.06 μm ± 0.143 S.E. The relative length of chromosomes ranged from 3.94 μm ± 0.051 S.E. to 14.06 μm ± 0.182 S.E. The karyotype and idiogram showed a gradual decrease in the length of chromosomes (Figs 2, 3).

E. lanigerum

The diploid chromosome number in *E. lanigerum* is 12 (Fig. 4). The chromosomes are holocentric. The mean length of the chromosomes ranged from 0.98 μm ± 0.011 S.E. to 3.39 μm ± 0.018 S.E. The mean total complement length is 27.62 μm ± 0.092S.E. The relative length of chromosomes ranged from 3.68 μm ± 0.054 S.E. to 12.29 μm ± 0.087 S.E. The karyotype and idiogram of this species showed a gradual decrease in the length of chromosomes (Figs 5, 6).

P. juglandis

The diploid chromosome number in this species is 22 (Fig. 7). The chromosomes are holocentric and the length of the chromosomes ranged from 0.69 μm ± 0.016 S.E. to 2.27 μm ± 0.024 S.E. The mean total complement length is 23.80 μm ± 0.118 S.E. The relative length of chromosomes ranged from 2.93 μm ± 0.016 S.E. to 9.55 μm ± 0.024 S.E. The karyotype and idiogram of this species showed a gradual decrease in the length of chromosomes (Figs 8, 9).



Figs 1–9: Cytology of aphids. 1–3. *B. amygdalinus.* 1. Somatic metaphase. 2. Karyotype. 3. Idiogram. 4–6. *E. lanigerum.* 4. Somatic metaphase. 5. Karyotype. 6. Idiogram. 7–9. *P. juglandis.* 7. Somatic metaphase. 8. Karyotype. 9. Idiogram. (Scale = 5 µm)

DISCUSSION

The diploid chromosome number in *B. amygdalinus* is 12, as reported by earlier workers (Talhouk 1977, Hussein & Qouar 1984, Blackman 1984). The chromosome number of 2n = 22 reecorded in the present study in *P. juglandis* is in conformity with the previous report of Blackman & Eastop (1994).

E. lanigerum constantly reproduces via parthenogenesis without involving sexual generations. Different morphs of this species showed variations in the diploid chromosome number (Gautam & Verma 1982). The diploid chromosome number is 12, and mean actual length of chromosomes ranged from 0.98 µm to 3.39 µm . Earlier, Gautam & Verma (1982) reported this range at 1.10 µm to 2.91 µm and Harper & MacDonald (1966) reported at 1.60 µm to 3.40 µm. Thus, the present study supports the view of Blackman (1980) that elimination of sexual generation produces karyotypic variations in the aphids as mentioned above.

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